small size of sample required, makes the method ideal for experimental animal investigations.

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HÆMATOLOGY

Incidence of the Blood Group Antigen Di^a in the Tuscarora Indians of North America

THE presence of the Di^a blood group antigen among various Indian tribes native to South and Central America has been well established, the incidence ranging from 3·7 to 45·8 per cent. Rare exceptions are the two documented groups where no Di(a+) examples have been demonstrated[1,2]. In North American Indians it has been found in 10·8 per cent of Chippewas of northern Minnesota[3], 8·6 per cent of Crees of northern Manitoba[4], 6·0 per cent of Bloods of southern Alberta[5], 0·35 per cent of Athabascans of Alaska[6], 5·6 per cent of Penobscots of Maine[7] and 0·00 per cent of Cherokees of Oklahoma[8].

The Tuscaroras were the last of the Six Nations to be admitted to the Iroquois League, the others being the Mohawks, Oneidas, Onondagas, Cayugas and Senecas. Their traditions assert that they are descendants of the original family of Iroquois leaving the parent group to migrate west to Lake Erie and then to the Mississippi River. Some of the band crossed the river and became lost to history. Those remaining on the eastern bank then travelled south and east to the Carolina sea-coast, settling in the region of the Neuse River. Driven out of this area by colonists and other Indians in the second decade of the eighteenth century, they were admitted to the Council of the Iroquois League in 1715 under sponsorship of the Oneidas and resided in the area bounded by the Unadella, Chenango and Susquehanna Rivers. Later, the Senecas gave land to the Tuscaroras in the western part of New York State where they reside at present on the Tuscarora Reservation north-east of Niagara Falls in Niagara County[9]. The present population of the Tuscarora Reservation represents some admixture principally with Whites and to a lesser extent with Indians of other nations.

Blood specimens were obtained from 53 unselected individuals living on the reservation. All samples were tested for the presence of the Di^a antigen using an anti-Di^a serum (*Woj*) produced by isoimmunization of pregnancy which resulted in hæmolytic disease of the new-born, this case representing the first example of the Di^a antigen and its antibody in a Caucasian family[10]. Of the 53 examined, six, or 11·3 per cent, were Di(a+). Interviews permitted only a division of the group into pure Tuscarora and those having admixture with other racial or Indian groups. Of the 34 considered to be full-blooded, three, or 8·8 per cent, were Di(a+); of the 19 in the admixture group, three, or 15·8 per cent, were Di(a+). The higher incidence in the latter group probably reflects its small size as well as the fact that sampling was not selected to exclude relatives.

Certain additional blood group determinations were carried out using anti-A, -A_1, -B, -D, -C, -E, -c, -e, -M,

Table 1. FREQUENCY OF ABO, MN, Rh, KELL AND DIEGO IN 53 RESIDENTS OF THE TUSCARORA INDIAN RESERVATION

	N	ABO System										MN System						Rh System								Kell System				Diego System			
		Phenotypes (per cent)						Genes (per cent)				Phenotypes (per cent)			Genes (per cent)			Phenotypes (per cent)								Genes (per cent)				Phenotypes (per cent)			
		A_1	A_2	A_1B	A_2B	B	O	A_1	A_2	B	O	M	MN	N	M	N		CDe	cDE	CDE	cde	ceddee	CCDee	CcDee	CCDEe	CcDEe	ccDEE	ccDEe	ceddee	K	k	KK	Kk	kk	K	k	Di(a+)	Di(a−)	Di^a	Di^b
Full blooded	34	50·0	0·0	0·0	0·0	5·9	44·1	29·5	0·0	3·0	67·5	47·0	41·2	11·8	67·6	32·4		55·9	33·8	0·0	10·3	2·8	32·4	2·9	0·0	44·1	5·9	11·7	2·8	0·0	100·0	0·0	100·0	0·0	100·0	8·8	91·2	4·5	95·5
Admixture	19	26·3	0·0	0·0	5·3	5·3	63·1	14·0	3·1	5·4	78·5	42·1	31·6	26·3	57·9	42·1		57·9	21·0	5·3	15·8	0·0	10·5	26·3	5·3	26·3	5·3	5·3	0·0	0·0	100·0	0·0	100·0	0·0	100·0	15·8	84·2	8·7	91·3
Combined	53	41·5	0·0	0·0	1·9	5·7	50·9	23·5	1·3	3·9	71·3	45·3	37·7	17·0	64·2	35·8		56·6	29·2	1·9	12·3	1·9	11·3	3·8	5·7	37·7	5·7	9·4	1·9	0·0	100·0	0·0	100·0	0·0	100·0	11·3	88·7	5·8	94·2

-N, -K and anti-Mi^a. The results of these examinations, together with those obtained using anti-Di^a, are presented in Table 1.

The incidence of 11·3 per cent Di(a+) individuals among the Tuscaroras of Western New York is the highest yet reported in Indians of North America.

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Direct ABO Group Determination of Bloodstains on a Micro Scale by Batch Procedure

The direct group determination of bloodstains in the ABO system is possible either by the mixed agglutination method[1,2] or the absorption-elution method[3–6]. Certain of these techniques[2,4,6] tend to be time-consuming because of the repeated individual manipulation of the materials being tested.

The technique described here enables grouping by both the mixed agglutination method and the absorption-elution method to be carried out on a micro scale by batch procedure. This is a distinct advantage in those methods which entail the microscopic investigation of agglutination results. A synthetic resin mountant is used to fasten bloodstained fibres to the wells of cavity slides. Advantage is taken of the discovery by Nickolls and Pereira[6] that prior fixation is unnecessary.

A fibre sample is obtained by shaving a blood-stained material with a razor blade or by cutting out a strand approximately 1–2 mm in length. The bloodstained fibres are teased apart with dissecting needles so that they are relatively free from one another. Although it is possible to test single fibres, it is not generally necessary to work with such a small sample: approximately 20 fibres can conveniently be handled with fine pointed forceps and a sample of this size is more frequently used in testing for each of the antigens A, B and H.

(1) Place a very thin smear (1–2 mm^2) of *MAC* mountant (G. T. Gurr, Ltd., London) in each of the three wells of a cavity slide (3 in. × 1 in., 12 mm wells). (2) Press the fibre sample lightly on to the surface of the *MAC* so that the fibres adhere without becoming occluded by the mountant. (3) Place in an oven at 50° C for 1–2 h to dry. (4) Dispense one drop of anti-A, anti-B and anti-H into the three cavities so that the fibre sample is completely covered. (A thin glass rod is useful for this purpose.) Allow to absorb for 1 h in a moist chamber. (A suitable chamber is described elsewhere[5].) (5) Continue absorption in a refrigerator at 4° C for 0·5 h. (6) Flood each slide individually with cold (4° C) saline to remove excess antibody and place in a stainless steel rack. (7) Wash by raising and lowering the rack 10–15 times in 300–400 ml. of cold saline. Place the rack in some clean, cold saline in a refrigerator for 10 min. Repeat the process of washing, remove the slides from the rack, blot dry with filter paper. (8) Dispense 30–40 μl. 0·1 per cent v/v A_1, B and O red blood cell suspensions into the relevant cavities. (9) Elute at 50°–55° C for 10–12 min in a pre-heated moist chamber. (10) Remove from the oven, rotate the slides, allow to cool, read the results after 15–20 min.

No interference due to the *MAC* mountant has ever been experienced: even so, an *MAC* blank is run in parallel with the tests, together with the appropriate fibre and positive controls.

Using a 0·1 per cent suspension of indicator cells, the results are typical of the absorption-elution method[3,7], positive results being indicated by the presence of free agglutinates of red cells in the suspension. If, however, a more concentrated cell suspension is used (for example, 1·0 per cent) the results are often typical of the mixed agglutination method. A positive result is indicated by the presence of red cells adhering to the blood-stained fibres in a cell suspension virtually free from agglutinates. The results using the higher concentration of cells have not been so consistently satisfactory, using normal antisera, as those obtained by using the lower concentration. I have had no opportunity of using immune antisera.

It is interesting to note that Ogata used a 2 per cent suspension and Coombs and Dodd a 1 per cent suspension of indicator cells. Nickolls and Pereira recommended 0·5 per cent, and stated in their results that there was heavy agglutination, some of which was adhering to the fibres. Kind advised the use of a 2 per cent suspension; but his original method was not a micro-technique. The quantity of antigen on test (and consequently also that of the eluted antibody) was much larger.

The results are most easily explained by considering the heterogeneous nature of the antibody populations. Goodman[8,9] and Kind[10] have shown that a human iso-antibody consists of a broad spectrum of closely related antibodies which vary in their degree of reactivity with the antigenic substances. Goodman suggests that this is due to their degree of complementarity with the antigens: certainly their avidity and reaction speeds vary. A stain saturated with antibody will release the substances with the smallest degree of reactivity first. Those antibodies which are more strongly reactive dissociate more slowly. In the micro test described here, using a low concentration of indicator cells, the antibody–antigen ratio is relatively high. The least avid antibodies, which dissociate first, elute and cause free agglutinates of red cells to form. The red cell receptors are either satisfied or blocked by the time the more avid antibodies are ready to react and consequently free agglutinates, typical of the absorption-elution test, are the only result.

When a more concentrated suspension of indicator cells is used, the antibody–antigen ratio is reduced. The fastest-eluting (that is, the least avid) antibodies are diluted by antigen to such an extent that either the agglutinates which form are very small or the indicator cells are only partially satisfied because of insufficient antibody being present to form agglutinates. The more avid antibodies, which dissociate to a much smaller degree, now combine with the red cells the receptors of which are only partially satisfied to form agglutinates attached to the bloodstained fibres. This result typifies the mixed agglutination test.

It will be appreciated that the indicator cell strengths given here will not necessarily be those required to give identical results in parallel tests with other antisera. The quantity of antibody eluted and its avidity are factors which must be determined empirically. Sera containing mainly avid antibodies will bias the test in favour of the mixed agglutination result: other sera containing a larger proportion of non-avid antibodies will favour the absorp-